CHAPTER 6

CONCLUSION AND FUTURE WORKS

6.1 Conclusion

Preliminary screening of lactic acid bacteria (LAB) with proteolytic activity was observed on skimmed milk agar (SMA) and the diameter of clear zones above 6 mm were considered as good proteolysis showed for LAB isolates Bd2, Pk2, WG2, and S1. These four LAB with high proteolysis generate whey skimmed milk with high antioxidative peptides compared to other LAB isolates via scavenging DPPH free radical activity and FICA assays resulted with the DPPH and FICA ranged between 12.59 and 19.04 %, and between 78.61 % and 86.95 %, respectively.

The isolates Bd2, Pk2, WG2, and S1 were further screened for their probiotic potentials and the results obtained for acid and bile challenges indicated that these LAB could survive in both environments with only slight reduction less than 1.05 log cycle. Antimicrobial activity for LAB isolates using dual culture overlay method showed inhibition of clear zone against *B. cereus* ATCC 10876, *B. subtilis* ATCC 21332, *S. aureus* ATCC 25923, *S. typhimurium* ATCC 13311, and *E. coli* ATCC 25922 ranged between 18.33 mm and 85.00 mm. While the antimicrobial activity for LAB isolates by agar well diffusion method against same targeted bacteria ranged between 8.00 mm and 13.83 mm. Thus, through acid and bile challenges as well as antimicrobial activity, these four proteolysis LABs also have potential as probiotic LAB hence these LABs were confirmed to maintain their viability and functionality. The same LAB of WG2, Pk2, S1, and Bd2 isolates were used as starter cultures in 24 h fermentation of buffalo milk to generate whey with antioxidative activity by different culturing approaches. The

scavenging DPPH free radical scavenging activity values of whey buffalo milk obtained by direct cultured LAB were higher than whey skimmed, and whey buffalo milk obtained by precultured LAB with range values between 22.61 and 33.62 %. The high FICA values of whey buffalo milk obtained by precultured LAB were lower than whey skimmed milk but higher than whey buffalo milk obtained by direct cultured LAB with range values between 59.41 and 78.22 %.

These LAB isolates of WG2, Pk2, S1, and Bd2 were identified as *L. plantarum* (WG2), *L. paracasei* (Pk2), *L. plantarum* (Sl), and *Enterococcus faecium* (Bd2), respectively. Fermentation of buffalo milk by these LABs were studied with different culture approaches at different fermentation times to generate whey with high antioxidative activity. The scavenging DPPH free radical scavenging activity values of whey buffalo milk by direct cultured LAB ranged between 21.87 and 55.03 % and FICA values of whey buffalo milk ranged between 50.13 and 65.52 % for both 24 and 48 h fermentation times. In contrast, the scavenging DPPH free radical scavenging activity values of whey buffalo milk by precultured LAB ranged between 3.43 and 12.28 % and the FICA values of whey buffalo milk ranged between 56.58 and 84.45 % for both 24 and 48 h fermentation times.

In this finding, the high antioxidative values obtained from whey buffalo milk generated by precultured *L. plantarum* WG2 and *E. faecium* Bd2 and were influenced by fermentation time. Precultured *L. plantarum* WG2 in 48 h fermented buffalo milk produced whey with high FICA values (84.45 %) and whey generated by precultured *E. faecium* Bd2 in 24 h fermented buffalo milk produced whey with second high FICA value (78.22 %). The half maximal inhibitory concentration (IC_{50}) by FICA for whey buffalo milk produced by precultured *L. plantarum* WG2 at 48 h fermentation was 0.37 mg/ml and whey buffalo milk produced by precultured *E. faecium* Bd2 at 24 h

fermentation was 0.39 mg/ml which was lower than IC_{50} value for standard EDTA (0.29 mg/ml).

From this study, the molecular weight (M_w) of these antioxidative peptides of whey buffalo milk produced by precultured *L. plantarum* WG2 and precultured *E. faecium* Bd2 was estimated between 150 and 50 kDa, and between 20 and 10 kDa which could be β-lactoglobulin (M_w = 80 kDa) and lactoferrin (M_w = 18 kDa) or could be other novel antioxidative peptides.

6.2 Future Works

This study revealed different LAB strains used as starter culture in both skimmed milk and buffalo milk may generate whey contained peptides with antioxidative activities. The LAB identified as *L. plantarum* WG2 and *E. faecium* Bd2 isolated from white grape and fermented salted fish can be applied to generate peptides from whey buffalo milk with relatively good antioxidant activity. In this study, selection of the LAB to generate antioxidative peptides from milk through fermentation should include proteolysis system and probiotic potentials as their most criteria have been achieved. Optimisation conditions for each LAB strains and different culturing approaches should be further studied to gather more data and to find suitable factors that will help to generate optimum antioxidative peptides of whey buffalo milk generated by these LABs. Band obtained by SDS PAGE in this study should further be evaluated to identify amino acid sequences responsible for peptides from whey buffalo milk with antioxidative activity. The selected areas of a gel containing selected bands can be processed for mass spectrometry (MS) such as liquid chromatography–tandem mass spectrometry (LC MS/MS) and continues with Blast for identification based on peptide sequence in Blast database.